

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

G. Barrie Kitto
Daniel C. Hirshhorn

Group Art Unit: 1638

Examiner: Cathy Kingdon Worley

Serial No.: 10/790,291

Atty. Dkt. No.: CLFR:232US

Filed: March 1, 2004

For: ANIMAL IMMUNOCONTRACEPTIVES
EXPRESSED IN PLANTS AND USES
THEREOF

CERTIFICATE OF ELECTRONIC SUBMISSION

DATE OF SUBMISSION: December 21, 2006

DECLARATION OF GEORGE BARRIE KITTO UNDER 37 C.F.R. §1.131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-01450

I, George Barrie Kitto, to declare that:

1. I currently hold the position of Professor in the Department of Chemistry and Biochemistry, College of Natural Sciences, at the University of Texas at Austin.

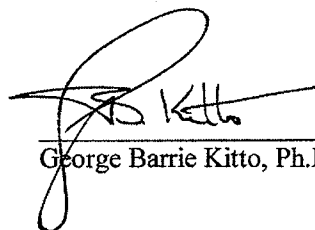
2. Attached are datasheets from the laboratory notebooks of Daniel C. Hirschhorn. These datasheets illustrate work that was carried out in my laboratory at the University of Texas at Austin prior to April 13, 2001.

3. Page 1 of the datasheets shows experiments using transgenic tobacco plants transformed with lactate dehydrogenase-C4 (LDH-C4). This datasheet shows results for the preparation of plant leaf extracts from these transgenic plants for the testing of these extracts for toxicity in mice. Also shown on the datasheet are results from an ELISA assay confirming the presence of LDH-C4 in the transgenic plant extracts. The transgenic plants expressing LDH-C4 were generated prior to these experiments.

4. Page 2 of the datasheets shows an experiment where extracts from recombinant tobacco plant leaves expressing LDH-C4 were prepared for contraceptive vaccine trials in mice. The transgenic plants expressing LDH-C4 were generated prior to this experiment.

5. I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

December 21, 2006
Date


George Barrie Kitto, Ph.D.

Preparation of extracts

For Toxicity

To determine the most adequate system for ~~the~~ plant preparation I did ~~an~~ ELISA and Westerns of the 3 types of prepare extracts. The OD₂₈₀ was to normalize total protein but since I will only doing toxicity test this experiment is not crucial in max CDH

OD₂₈₀ → to determine approx ~~value~~ [] to equalize for ELISA

#1 .450 → spin more 30 min = 7

#2 .754
.800 .8

#3 .33 → spin more 30 min .6
.34

WT = .04 → spin more 30 min

ELISA

	1	2	3	4 wells
A	0	0	#1 1	1 wt
B	.5	.5	#1 1	1
C	1	1	#1 1	5
D	2	2	#2 1	5
E	4	4	2 1	10
F	6	6	2 1	10
G	8	8	#3 1	
H	10	10	1	
	1/100	1/200	1	

Also the frozen stock gave no rxn → do not use frozen stuff

	01	02	03	04
A	0.175	0.187	0.599	0.185
B	0.558	0.999	0.585	0.144
C	0.474	0.929	0.583	0.141
D	0.655	0.816	0.711	0.157
E	0.855	1.073	0.743	0.160
F	0.949	0.929	0.751	0.148
G	1.126	1.064	0.122	0.207
H	1.179	1.105	0.128	0.211

$$.150 \times 5 = .75 \text{ ng/ml}$$

so I get 225 ng in 300 µl

First PLANT EXTRACTION FOR VACCINE TRIALS

For this purpose and since I'm leaving to Mexico I decided to do an acetone powder extraction.

Take 100 grms of leaf (if possible) and ~~an~~ grind in LN₂ to a fine powder.

Transfer powder to a blender ~~and~~ containing ~100ml of 1X PBS w/ 1 tablet of protease inhibitors and 2X PMSE and grind until a homogenate is formed.

Filtered through a cheese cloth and depending on how chunky things are filtered again using a filter paper or a wire mesh.

Add 5x volumes of Acetone and keep at -20 °C for several hours.

I just filtered through a cheese cloth.

recovered 3.4 grms of powder dry